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***In Vivo* Ibogaine Blockade and *In Vitro* PKC Action of Cocaine^a**

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ABSTRACT: Ibogaine may have antiaddiction potential against alcohol, psychostimulant and opiate abuse, but its mechanism of action is unclear. Ibogaine, however, has been demonstrated in numerous studies to have effects in multiple central nervous system (CNS) neurotransmitters systems. We are using *in vitro* and *in vivo* systems to study the effects of cocaine and whether these effects can be blocked by ibogaine. For the *in vivo* studies, we first determined the acute and subacute effects of ibogaine (1–5.0 mg/kg) in mice using the plus-maze test. Acutely increasing doses of ibogaine produced a reduced aversion to the open arms. The subacute administration provoked a variable response which was characterized by fluctuations in aversive and antiaversive behavior of the animals to the open arms of the plus-maze during the 14-day treatment period. A separate group of mice received 1.0 mg/kg cocaine for 14 days, and upon abrupt cessation from cocaine treatment, ibogaine 2.5 mg/kg was administered to a subgroup of these mice. Ibogaine reversed the withdrawal aversions produced by the abrupt cessation from cocaine administration. For the *in vitro* studies, the expression and activity of protein kinase C (PKC) isoforms and Ca²⁺ levels were examined following the incubation of PC 12 cells with cocaine. This is because PKC plays a key role in a number of cellular and neuronal functions. We report that cocaine disrupts signal transduction in PC 12 cells by altering the expression and activity of PKC isoforms and Ca²⁺ levels. The data obtained suggest (1) that the PC 12 cells may be useful in studying the neurobiology of abused drugs, like cocaine *in vitro*, (2) that if anxiety is a factor in drug dependency, then the antiaddictive property of ibogaine *in vivo* may be associated with modifying the CNS neurotransmission that may be involved in anxiety. It remains to be determined whether the signaling involving PKC is important in the antiaddictive properties of ibogaine.

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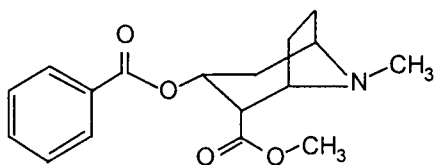
INTRODUCTION

Ibogaine: Beyond the Nucleus Accumbens and Dopamine Hypothesis of Reward

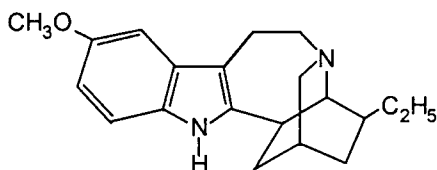
Long-term use or ingestion of cocaine, amphetamine, heroin, benzodiazepines, nicotine in smoking, caffeine, alcohol, and marijuana may cause addiction with craving and withdrawal syndrome acting as a deterrent from quitting or cessation of drug and alcohol use. The central dopamine hypothesis of reward and reinforcement in drug addiction has been associated with elevated dopamine levels in the nucleus accumbens (Acb), which has therefore been suggested as the central hub and final common neuroanatomical target for abused drugs in the brain.¹⁻⁵ If the dopamine hypothesis of reward and the Acb brain structure associated with drug reinforcement is all but proven, then manipulation of the mesocorticolimbic dopamine system should provide medications to treat drug and alcohol dependency in the clinic. However, effective drug abuse treatment continues to be elusive. The efficacies of new treatments for drug abuse has been largely disappointing and new therapies are needed. Different approaches, including an immunological approach to cocaine addiction, have been described.⁶ There are doubts of the effectiveness of cocaine immunogen and whether or not it will be effective in blocking human addiction to cocaine.⁷ Therefore, there is now more than ever a need to develop new thinking and hypotheses to aid our understanding of the mechanisms leading to drug dependency.

While it has been previously recognized that the reward centers in the brain consists of multiple systems and neuroanatomical sites, emerging data have started to challenge the dopamine hypothesis of reward involving the Acb circuitry.⁸ The recent studies in normal and cocaine addicts using positron emission tomography (PET) are associated with metabolic abnormalities in orbitofrontal cortex and the striato-thalamic-orbitofrontal circuit, which has now been postulated as a common mechanism underlying drug and alcohol addiction.⁸⁻¹¹ Furthermore, there are other dopamine-independent mechanisms involving other neurotransmitters like glutamate,^{12,13} γ -aminobutyric acid (GABA), dynorphin, serotonin (5-HT) and cholecystokinin (CCK) not only in the Acb but in other brain regions like the frontal cortex, hippocampus, locus coeruleus, lateral hypothalamus or the periaqueductal gray that are potential neural substrates for the rewarding properties of psychostimulants, benzodiazepines, barbiturates, opiates and phencyclidine hydrochloride (PCP).^{6,14} Since the usefulness in treating any addiction with dopaminergic agents has been limited,^{15,16} one emerging potential yet controversial therapeutic agent is ibogaine, an indole alkaloid isolated from the bark of the African shrub, *Tabernanthe iboga* (FIG. 1).

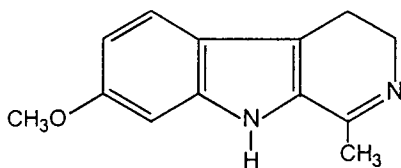
There are claims that this naturally occurring alkaloid may have antiaddiction potential, and that ibogaine can be used in the treatment of drug abuse by suppressing the desire and reducing craving and withdrawal symptoms associated with heroin, psychostimulants and alcohol addiction in humans and animal models of drug abuse.¹⁷⁻²¹ The mechanism of action associated with the ability of ibogaine to block drug-seeking behavior is currently unknown, and a number of studies suggest that ibogaine has a broad spectrum of action on multiple systems.^{19,22} It is speculated that this broad spectrum of activity on opiate, serotonin, dopamine, choline, glutamate (*N*-methyl-D-aspartate (NMDA)), sigma, noradrenergic and other hormonal systems may in part contribute to the putative antiaddictive properties of ibogaine. As it is now doubtful that the mesolimbic dopamine acts by directly producing feelings of pleasure or euphoria,²³ we have to move beyond the nucleus accumbens and dopamine hypothesis of reward. In the place of the dopamine hypothesis our working hypothesis is that the molecular events that underlie the development of compulsive drug-seeking behavior involve multiple brain sites and systems in drug reinforcement. These molecular switches



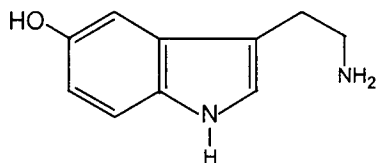
Cocaine



Ibogaine



Harmaline



Serotonin

FIGURE 1. Chemical structures of cocaine, ibogaine, harmaline and serotonin.

lead to neuroplastic alterations in specific signal transduction systems that turn on/off subsets of genes that precipitate the behavioral manifestations of loss of control and compulsive drug or alcohol use. Alcohol and abused drugs turn on the switch and withdrawal, craving or relapse turns off the switch that creates the neuroadaptive addiction cycle. It is therefore possible that ibogaine through its multiple actions can restore the hedonic homeostatic dysregulation caused by drug and alcohol abuse.²⁴

In these studies we have used *in vitro* and *in vivo* systems to study the effects of cocaine and whether these effects can be blocked by ibogaine.

IBOGAINE BLOCKS THE ACTION OF COCAINE *IN VIVO*

For many years the powerful reinforcing effects of psychostimulants, including cocaine and other abused drugs, have been linked to the mesocorticolimbic dopamine system and its connections.¹⁻⁵ Although, dopamine is still thought to play a critical role in motivation and reward, it is now doubtful that the mesocorticolimbic dopamine acts directly as the brain reward center.²³ As discussed by Wickelgren recently, "Rather than signaling pleasure as previously thought, the neurotransmitter dopamine may be released by neurons to highlight significant stimuli."²³ The neurobehavioral effects of cocaine may be linked to a number of factors including the route of administration, dose of cocaine, the environmental cues and coadministration of other substances including alcohol. It is unlikely that the overall neurobehavioral effects of cocaine are due to a single neurotransmitter action in one pathway in the central and peripheral nervous system, but it is more likely that they are the result of the effect on multiple systems. The broad spectrum of action of ibogaine, therefore, made it attractive to investigate whether it will block an *in vivo* action of cocaine that is linked to emotionality/stress, which may be a factor in drug dependency.

Institute for Cancer Research (ICR) mice used in this study were housed in groups of five. Average weight varied from 18–25 g. Food and water was available *ad libitum*. Cocaine and ibogaine were made up in normal saline. For the *in vivo* studies we first determined the acute and subacute effects of ibogaine (1.0–5.0 mg/kg) in mice using the elevated plus-maze test. For the antagonism studies, a separate group of mice received 1.0 mg/kg cocaine for 14 days and upon abrupt cessation from cocaine treatment, ibogaine, 2.5 mg/kg was administered to a subgroup of these mice. The performance of all the treated mice was further evaluated in the plus-maze 1, 2, 3 and 4 days after withdrawal from drug or vehicle administration. The plus-maze was made of two open arms and two enclosed arms that were all linked by a central platform, and arranged such that the arms of the same type were opposite each other. The performance of each animal in the maze was initiated by placing each animal in the center of the plus-maze, with the head facing an open arm. The number of entries and amount of time spent in the open arms, enclosed arms and the central platform were recorded during the 5-min test sessions.

FIGURE 2a–c shows the acute and subacute effects during treatment and withdrawal from ibogaine on the performance of ICR mice in the elevated plus-maze test. There was no clear dose-response profile of action of ibogaine following the acute administration of ibogaine. The data obtained following treatment with ibogaine or the combination with cocaine was compared to the effect of vehicle-treated control mice. While the decrease in time spent in the enclosed arms at the lowest dose of 1.0 mg/kg may indicate an antiaversive action, the 2.5-mg/kg dose induced an aversive action in the open arms characterized by a decrease in the time spent in these arms. Ibogaine at the doses used had no significant effect on the time spent in the central platform. The number of entries into the open and enclosed arms was reduced only at the 2.5-mg/kg dose acutely

(data not shown). At the highest dose of ibogaine used, the time spent and number of entries into the open and enclosed arms and the central platform were not different from controls. The repeated treatment of mice with ibogaine induced aversive and antiaversive behavior to the open arms on day 4 and day 13, respectively. An antiaversive behavior of mice injected with the 5-mg/kg dose was also recorded on day 10 as shown by the reduced time spent in the enclosed arms. This subacute treatment with ibogaine did not affect the time the animals spent in the central platform (FIG. 2c). Upon withdrawal from the 14-day treatment with ibogaine, there were no differences in the time spent and number of entries into the open arms, enclosed arms and central platform in comparison to control animals.

The influence of ibogaine 2.5 mg/kg on cocaine withdrawal in the plus-maze test is shown in FIGURE 3a–c. Upon withdrawal from cocaine 1.0 mg/kg, ibogaine was administered daily for 4 days accompanied by the daily assessment of the plus-maze performance. Cocaine induced mainly an antiaversive profile with increase in time spent in the open arms. The withdrawal from cocaine administration induced intense aversion to the open arms by day 2. This intense aversion was blocked by ibogaine. The behavioral data obtained adds to the growing evidence that ibogaine, its congeners and perhaps its metabolites may have value in the treatment of drug and alcohol dependency. This is supported by other animal and human anecdotal and clinical evidence.²² Although, there are some negative data, a number of the animal works indicate that ibogaine reduces some of the behavioral manifestations associated with cocaine administration and withdrawal.²² For example, in a study in mice, ibogaine reduced cocaine consumption in a drinking preference model.²⁵ In another mouse study, ibogaine did not reduce the withdrawal manifestations following naloxone-precipitated withdrawal in morphine-dependent mice.²⁶ In rats ibogaine has been shown to decrease morphine self-administration,^{27,28} reduce the frequency of withdrawal symptoms induced by naloxone,^{27,29} and decrease intravenous cocaine self-administration.³⁰ The effects of ibogaine on alcohol consumption has also been investigated in animals, and it has been demonstrated that ibogaine and one of its metabolites, noribogaine, reduces alcohol consumption in a number of alcohol-preferring rat lines.^{21,31,32} There are suggestions that the use of ibogaine in the treatment of drug and alcohol abuse be viewed with some degree of caution²⁰ because of its hallucinogenic properties and perhaps toxicity, but it is difficult to ignore the balance of evidence now emerging from animal and human data. In a preliminary study of seven individuals addicted to opiates, three who had at least 1.0 g ibogaine had remained drug free for 14 weeks.³³ Therefore, there is some merit in further investigation of the value of ibogaine in drug and alcohol dependence, which may form a template for the development of novel compounds for substance abuse pharmacotherapy.

While the neuronal and molecular basis for the antiaddiction properties of ibogaine, its congeners and its metabolites are incompletely understood, it has been postulated that these ibogaine-like compounds may be countering the multiple actions of alcohol and abused drugs.²¹ Most drugs of abuse and alcohol are known to have effects and interactions with one or more of these multiple systems, including activation of gene expression, 5-HT, dopamine, GABA, glutamate, noradrenergic opiates and hormones, particularly stress hormones.²¹ Because of the promiscuity of action of ibogaine, it is not surprising that it has shown promise preclinically and clinically. Thus, several approaches including pharmacological, behavioral, radioligand binding, toxicological, histochemical, biochemical, histological, spectrometry and synthesis has been used to probe the action of this naturally occurring alkaloid. Overall consensus data support the multiple effects of ibogaine. An area of void in these studies has been what genetic and transcription factors are important in the multiple actions of ibogaine. As hypothesized above, ibogaine may be switching off a subset of genes that have been

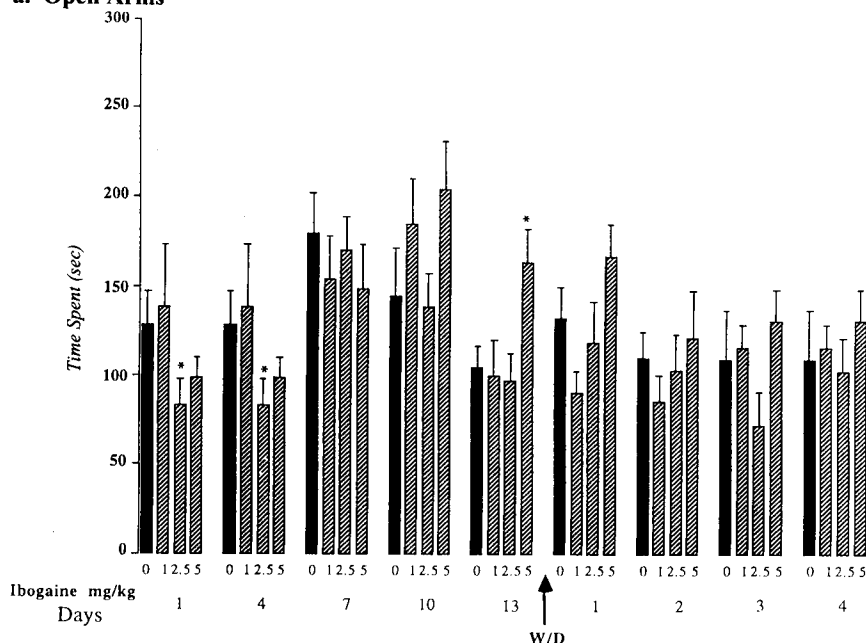
a. Open Arms

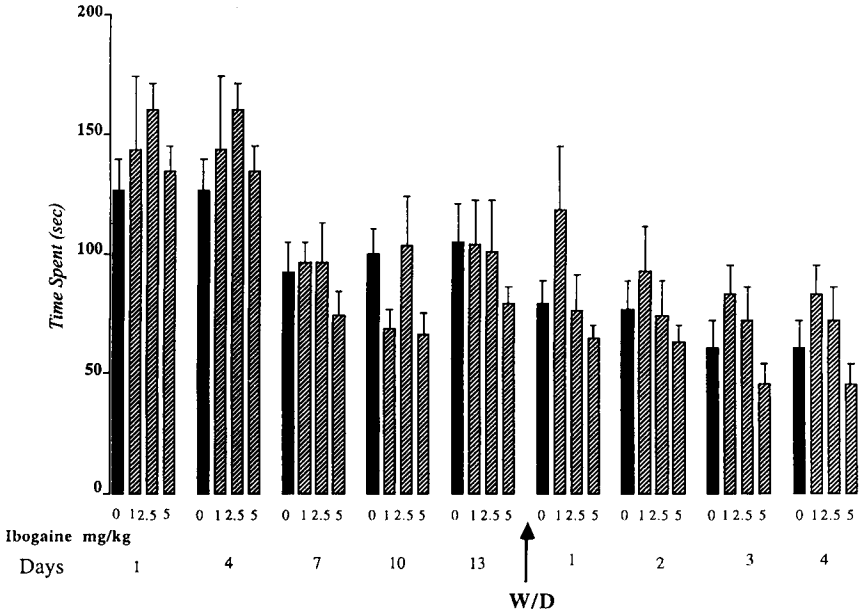
FIGURE 2. The acute and subacute effects during treatment and withdrawal (W/D) from ibogaine on the performance of ICR mice in the elevated plus-maze test. The time spent (sec) in the (a) open arms, (b) central platform, and (c) enclosed arms of the plus-maze following the 5-min test session are shown. Differences between the vehicle (0) and ibogaine-treated animals are indicated as * $p < 0.05$ (one-way analysis of variance (ANOVA) followed by Dunnet's t test).

turned on by alcohol and abused drugs. One transduction cascade that has been associated with chronic administration of opiates or psychostimulants is the cyclic adenosine monophosphate (cAMP) signal transduction pathway which leads to cAMP-responsive element-binding protein (CREB) phosphorylation and downstream gene expression that in principle can be regulated by protein kinases.^{34,35} We have started to investigate the role of protein kinase C (PKC) in the actions of alcohol and abused drugs, and future studies will determine whether or not PKC plays any role in drug and alcohol dependency and whether or not such actions can be blocked by ibogaine.

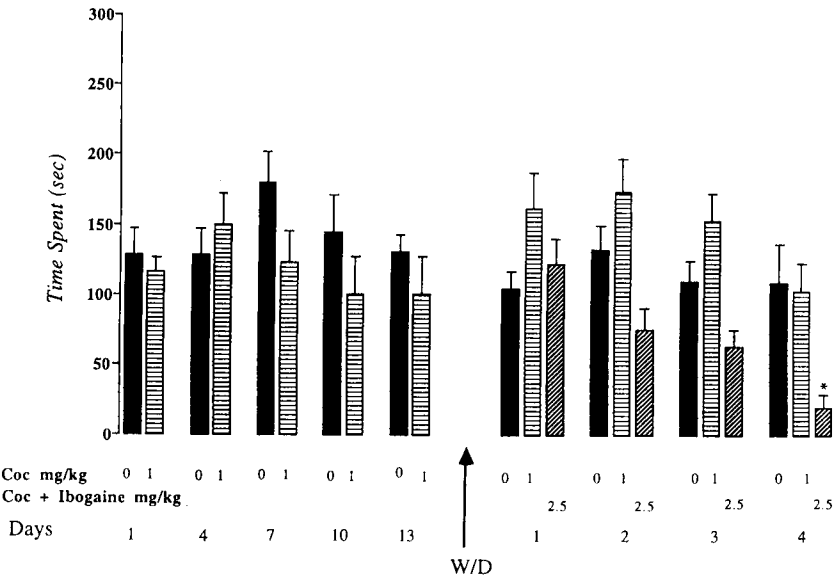
IN VITRO PKC ACTION OF COCAINE

As discussed above, the neurobiological processes leading to alcohol and addiction to drugs like cocaine are not well understood. The matter is further complicated by the withdrawal syndrome and relapse that occurs during attempts to quit the use of alcohol and most of the abused drugs like cocaine. PC 12 cells have been utilized by a number of investigators to study the effects of ethanol but not that of cocaine on cell function.³⁶⁻³⁸ Thus, chronic ethanol exposure has been shown to increase protein kinase C

b. Central Platform



c. Enclosed Arms



a. Open Arms

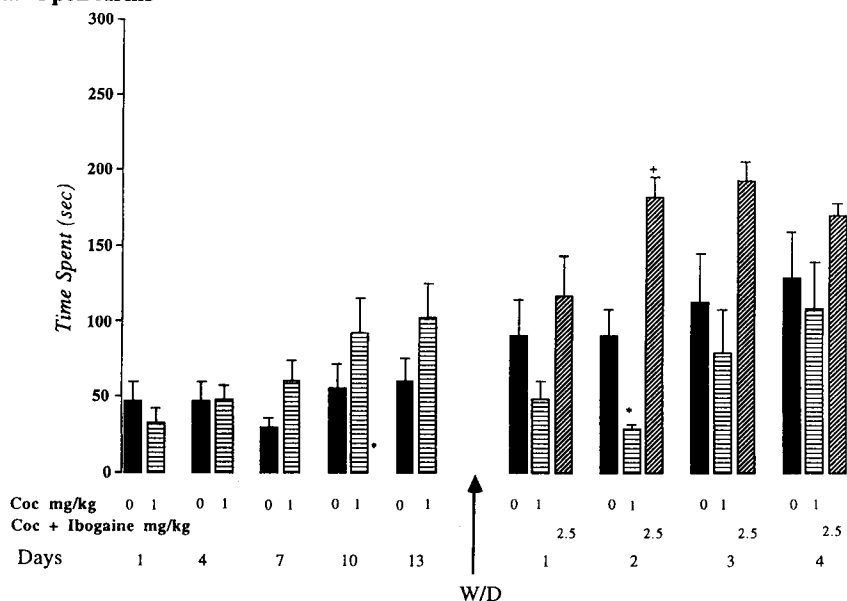
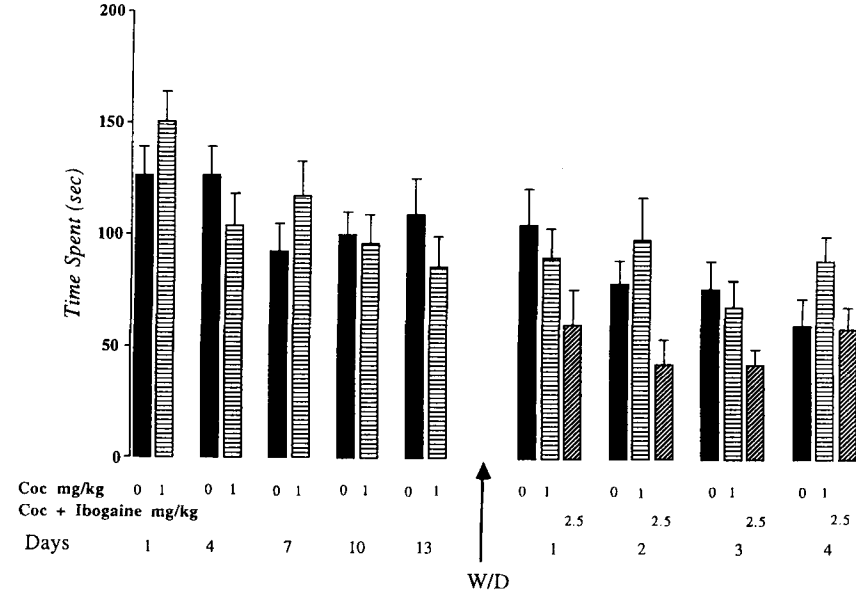


FIGURE 3. The influence of ibogaine 2.5 mg/kg on cocaine withdrawal in the elevated plus-maze test. Upon withdrawal (W/D) from cocaine (1.0 mg/kg), ibogaine (2.5 mg/kg) was administered daily for 4 days accompanied by daily assessment of plus-maze performance. The time spent (sec) in the (a) open arms, (b) central platform, and (c) enclosed arms of the plus-maze following the 5-min test session are shown. Significant differences from vehicle-treated animals are indicated as * $p < 0.05$ or + $p < 0.05$ (one-way ANOVA followed by Dunnett's t test).

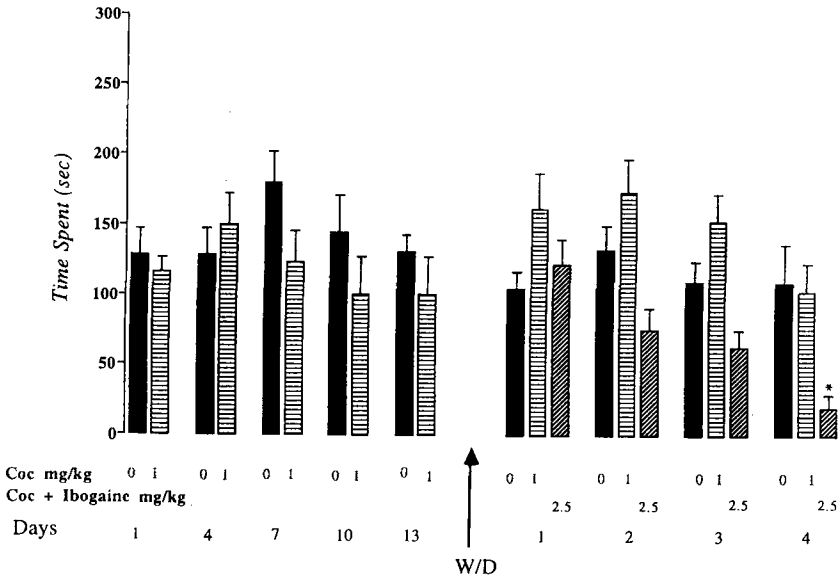
activity and regulation of Ca^{2+} channels in PC 12 cell lines by these investigators. The PC 12 clonal cell line of neural crest origin possesses the ability to store and secrete dopamine and other neurotransmitters that are known to be affected by cocaine. These cells are coupled to second messenger systems necessary for signal transduction in response to a variety of stimuli. PKC consists of a family of closely related isoforms, which differ in their localization and pharmacological properties. It is a major mediator of transducing signals to the interior of the cell, and it is activated *in vivo* by Ca^{2+} and diacylglycerol. The activity and translocation of PKC has been implicated in a number of cellular and neuronal functions. Previous studies have therefore suggested a role of PKC in the modulation of ethanol effects on receptor function in cells of central nervous system (CNS) origin. The aim of the present study was to determine the activity and expression of PKC isoforms along with changes in Ca^{2+} levels following incubation of PC 12 cells with cocaine. While alcohol-induced increases in PKC levels have been associated with the upregulation of Ca^{2+} , we demonstrate for the first time the ability of cocaine to disrupt signal transduction in PC 12 cells. A preliminary report of these data has already appeared.⁴⁷

Stock culture of rat pheochromocytoma cells were obtained from American Type Culture Collection (Rockville, MD). The cells were maintained in Corning 75 cm^2 canted neck, plug seal capped flasks (Corning, NY), and incubated in a humidified at-

b. Central Platform



c. Enclosed Arms



mosphere containing 5% CO₂ at 37°C. PC 12 cells were fed a Roswell Park Memorial Institute (RPMI)-1640 85% medium (Sigma Chemical Co., St. Louis, MO) supplemented with heat-inactivated (30 min, 56°C) 5% horse serum (Sigma Chemical Co., St. Louis, MO) and 10% fetal bovine serum (Biocell Labs., Inc., Rancho Dominguez, CA). The RPMI contains 1% L-glutamine. The medium was changed every 2–3 days. The cells were routinely passaged before they reached confluence, and subcultured by re-seeding at a density of about 1×10^5 cell/ml in the T-75 flasks. The cells were counted and the viability was assessed as previously described.³⁶ The preparation of the cultured cells and incubation with cocaine was performed as previously described with slight modification.^{36,38} Several concentrations of cocaine (0.001–7.5 mM) were incubated with semiconfluent PC 12 cells from 4 hr to 6 days. The medium was changed once during the incubation period, and the treatment of the cells with cocaine was repeated. At the end of the treatment period the PC 12 cells were rinsed in ice-cold PBS buffer and the intact cells after incubation were transferred into a glass homogenizer and homogenized. Following the estimation of the protein content, the cell homogenate were further prepared for immunoblotting analysis, using standard procedures^{36,38,40} with slight modification. Equal quantities of protein samples from the cocaine-treated cells were fractionated electrophoretically on the sodium dodecylsulfate polyacrylamide gels (1%) in duplicates. This is to allow for Coomassie blue staining of the protein in one gel and the duplicate transferred onto the nitrocellulose membrane (BioRad, USA). After the transfer, the nitrocellulose membranes were placed in a 1% nonfat milk blocking solution containing 50 mM tris(hydroxymethyl)aminomethane (TRIS), pH 7.5, 0.15 M NaCl, 0.05% Tween 20 by rocking the blots at room temperature for at least 1 hr. Blots were incubated overnight with the PKC subtype antibodies, monoclonals against the α , β and γ (Gibco BRL) and polyclonals against δ , ϵ and ζ (Sakaguichi, MD). These antibodies were detected using alkaline phosphatase-conjugated goat anti-mouse or goat anti-rabbit immunoglobulin G (IgG) and nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) (Gibco BRL). The activity of PKC in the cell homogenate following the six-day incubation of the PC 12 cells with cocaine was measured by the method of Gopalakrishna *et al.*,⁴¹ with slight modification using the Millipore multiscreen system. PKC substrate solution (1mM CaCl₂, 20 mM MgCl₂, 20 mM TRIS pH 7.5, 20 μ M adenosine triphosphate (ATP), histone (0.25 mg/ml) 20 μ g/ml leupeptin and [γ -³²P] ATP) was prepared. 25 μ l of the samples to be assayed were added to the wells of a 96-well filtration plate used for the PKC assay. 50 μ l of 20 mM ethylene glycol-bis-(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) or PKC inhibitor solution (100 μ M PKC (19–36)) was added to the control wells. To the remaining wells 50 μ l of a lipid preparation (100 μ l phorbol-12-myristate-13-acetate (PMA), 2.8 mg/ml phosphatidyl serine, Triton X-100 mixed micelles) was added. The plates containing the samples and the PKC substrate solution were then prewarmed to 30°C in an incubator for 30 min. The reaction was then initiated by adding 125 μ l of the PKC substrate to each well, and the samples were incubated for 5 min at 30°C. The reaction was then terminated by adding 75 μ l of 70% trichloroacetic acid (TCA) to each well and gently shaking, 150 rpm for 5 min at 4°C. The TCA was then drained from the wells. The filters were subsequently washed 6 times with ice-cold 25% TCA. The filters were then dried with a heat lamp and punched from the plates into individual scintillation vials containing a scintillation cocktail, shaken for 3 min and the radioactivity counted. The PKC activity was calculated and expressed as pmol/mg protein. All of the samples were analyzed for protein content using bovine serum albumin as the standard. Data are expressed as mean $\pm$ SEM. Statistical difference was calculated by Student's *t* test for single comparison or one-way analysis of variance combined with Dunnett's *t* test for multiple comparison. Levels of significance was determined when the *p* value was less than 0.05.

Treatment of PC 12 cells with cocaine (0.01–3.0 mM) modified the activity and expression of the PKC isoforms and increased the intracellular levels of Ca^{2+} in the cells. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting analysis of the PC 12 cell homogenates with antibodies against PKC, α , β , γ , δ , ϵ and ζ after incubation with doses of cocaine are shown in FIGURES 4 and 5. The spectrum of the effects of increasing doses of cocaine varied according to the isoforms. At doses up to 7.0 mM cocaine was lethal to the PC 12 cells and inhibited the expression of all the isoforms examined (data not shown). There was inhibition of the expression of PKC α at the low dose of 0.01 mM and increased expression at higher doses (0.1–3.0 mM) of cocaine. Immunoblotting with the anti-PKC β antibody detected an 80-kDa protein, whose expression increased as the dose of cocaine increased. While the expression of PKC δ also increased with increasing doses of cocaine, the expression of PKC γ and PKC ϵ decreased with increasing doses of cocaine, with the expression of PKC ζ remaining significantly unchanged. The incubation in the presence or absence of the antigenic peptides allowed identification of the PKC isoforms by the occurrence of immunolabeled bands, which were not seen when antigenic control peptide was present (Fig. 4, lane 6). Interestingly, the total activity of PKC increased with increasing concentrations of cocaine and declined with concentrations above 3.0 mM when compared to PC 12 cells that were not exposed to cocaine, as shown in FIGURE 6. The levels of Ca^{2+} in the PC 12 cell homogenates with or without incubation with cocaine were measured using fura-2 and analyzed with the SPEX AR-CM fluorometer. The Ca^{2+} levels significantly increased with increasing concentrations of cocaine in the PC 12 cells compared to the controls, as shown in TABLE 1. These data, therefore, confirm that the antibodies used can detect PKCs, α , β , γ , δ , ϵ and ζ in the PC 12 cells and show that cocaine differentially affects the expression of the subtypes of protein kinase C.

These results demonstrate for the first time the ability of cocaine to affect the activity and expression of PKC isoenzymes in PC 12 cells. The effect of cocaine was dose dependent and specific for the different isoforms of PKC. The differential expression of the PKC isoforms was followed by increased total PKC enzyme activity and Ca^{2+} levels. These effects of cocaine in the expression and activity of PKC in the PC 12 cells share some similarities and differences with the results previously obtained with ethanol in PC 12 cells.³⁸ It was demonstrated that chronic ethanol exposure increased the levels of PKC δ and ϵ and PKC-mediated phosphorylation in cultured neural cells.^{36,38} Our results indicated that, like ethanol, cocaine increased the levels of PKC δ , but, unlike ethanol, increasing doses of cocaine decreased the expression of PKC ϵ . Furthermore, a number of studies have suggested a role of PKC in the modulation of ethanol effects on receptor function in cells of CNS origin, and the bases of some of these pharmacological effects may be related to the PKC-derived transduction mechanisms.^{37,42,43} PKC activity has also been linked with tolerance to the effects of alcohol³⁸ and morphine.⁴⁴ In the CNS, alcohol and cocaine are known to disrupt a number of hormonal and neurotransmitter systems, including dopaminergic mechanisms that may be asso-

TABLE 1. $[\text{Ca}^{2+}]_i$ Measurements in PC 12 Cells Using Fura-2

Treatment	$\Delta [\text{Ca}^{2+}]_i$, nM	% Control
Control	43.70 ± 5	100
0.01 mM cocaine	349.30 ± 25	799
0.10 mM cocaine	270.40 ± 19	619
1.0 mM cocaine	19273.0 ± 2001	44103
3.0 mM cocaine	21137.0 ± 1998	48368

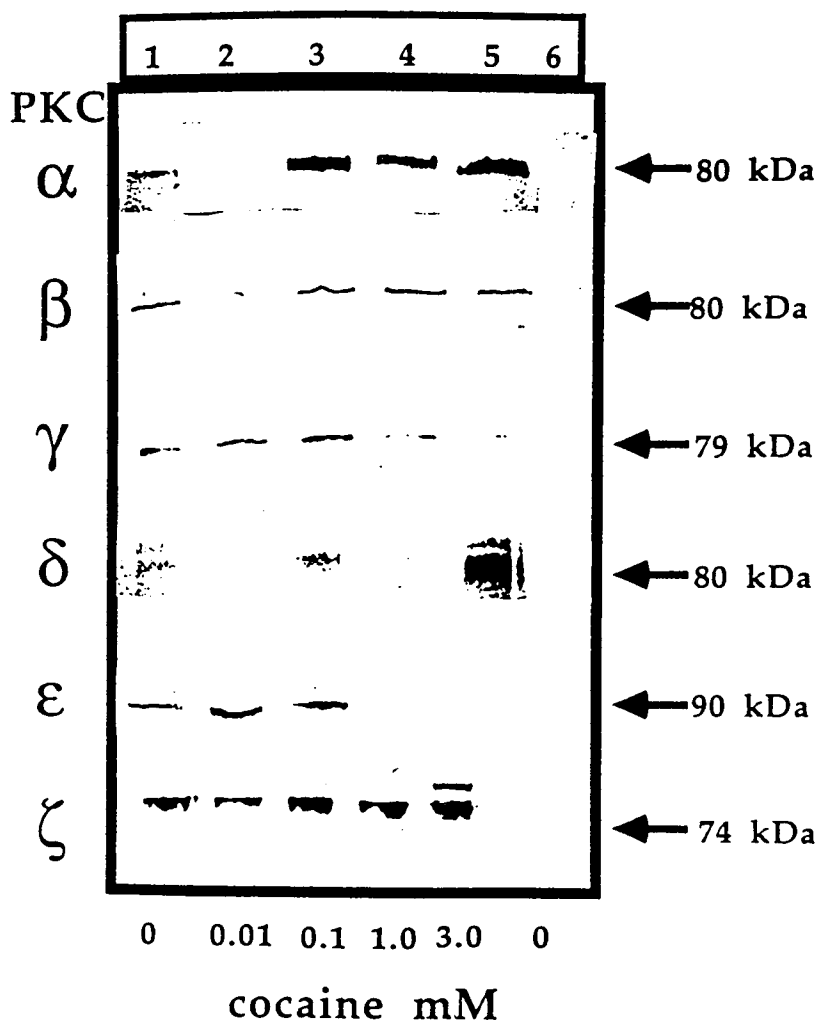


FIGURE 4. Differential expression of PKC isoforms and specificity of anti-PKC anti-bodies. PC 12 cells were treated with cocaine (0.01–3.0 mM) for 0–6 days. Cells were harvested as described and analyzed by SDS-PAGE/Western blotting with isoform-specific antibodies to α , β , γ , δ , ϵ or ζ PKC isoforms. Lanes 1–5 indicates doses of cocaine and lane 6 is the control PC 12 cells incubated with antibody/peptide complex. The various molecular weights of the isoforms detected are shown. These data are a representative of five independent determinations. The quantitative data of the results is shown in FIGURE 5.

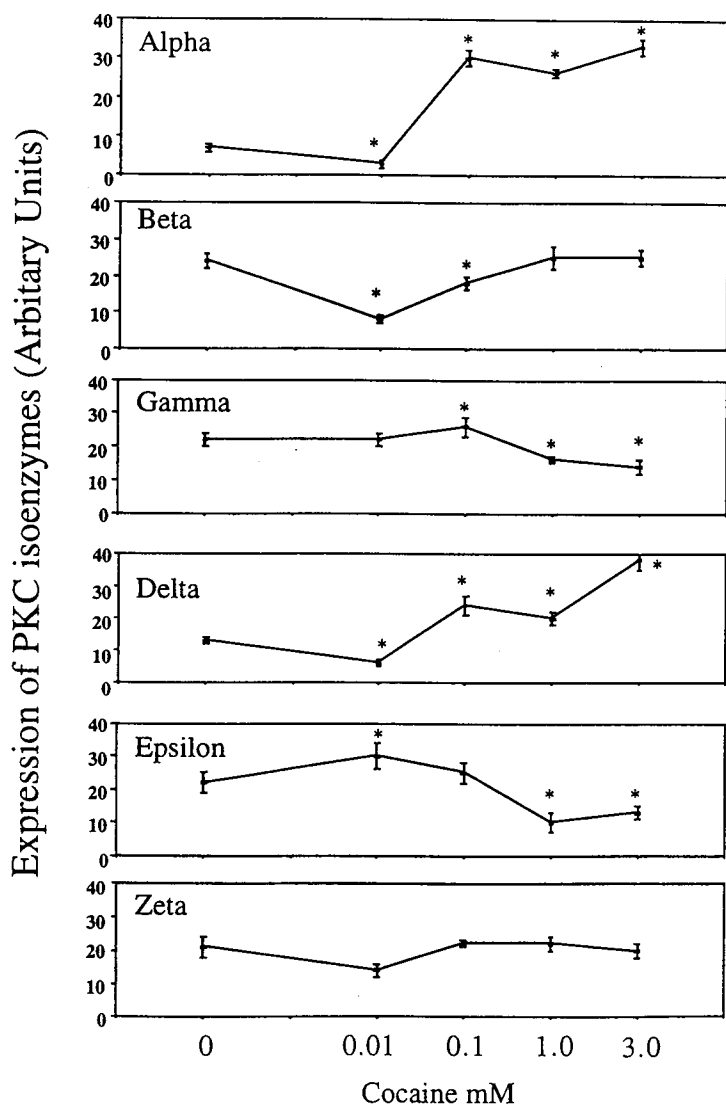


FIGURE 5. The differential expression of PKC isoenzymes in arbitrary units was determined by IS-1000 Digital Imaging system (Alpha Inn-Tech Corporation). The immunoblots were scanned and quantified. The plots represent the mean $\pm$ SEM for the five independent blots. The levels of significance are indicated as $*p < 0.05$ for differences between PC 12 cells treated with cocaine and controls (one-way ANOVA followed by Dunnett's t test for multiple comparison with vehicle).

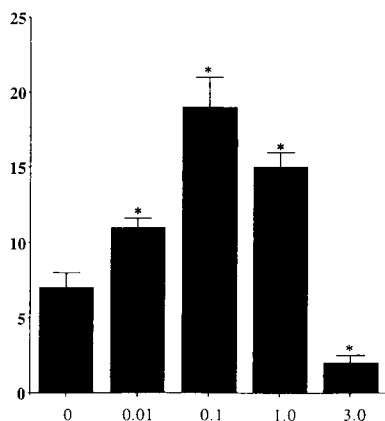


FIGURE 6. Total protein kinase C activity in homogenates of PC 12 cells. The PC 12 cells were treated with or without cocaine for 6 days and the total activity in the homogenates analyzed as described. All the samples were analyzed for protein content using bovine serum albumin as standard. The assays were performed in triplicates. The levels of significance are indicated as $*p < 0.05$ for differences between PC 12 cells treated with cocaine and controls (one-way ANOVA followed by Dunnett's t test for multiple comparison with vehicle).

ciated with compulsive alcohol and drug use and relapse. Since not all the physiological actions of the multiple dopamine systems can be explained by the modifications of the cAMP-dependent pathway, some studies have suggested an involvement of the inositol phosphate-generating system.⁴⁵ There is increasing experimental evidence for the existence of cross-talk or interaction between multiple signal transduction systems^{40,45} in the action of drugs that modulate the dopamine system, including psychostimulants like amphetamine. The data obtained in this study suggest some role of PKC in the effects of cocaine and lends further support to the probable existence of cross-talk between multiple signal transduction systems.

It is known that PKC is a soluble enzyme in its active state, and translocation to the plasma membrane is required for its activation by Ca^{2+} and phospholipids.⁴⁶ However, not all PKC isoenzymes are Ca^{2+} dependent. It was not surprising that the levels of Ca^{2+} were increased by the treatment with cocaine, because any one or a combination of the following mechanisms can be speculated to be involved: 1) by increasing Ca^{2+} channels as reported for ethanol;³⁸ 2) by inhibition of the plasma membrane Ca^{2+} ATPase pump, endoplasmic reticulum Ca^{2+} pump, mitochondrial Ca^{2+} uptake; and/or 3) due to the stimulation of the release of Ca^{2+} from internal storage by opening intracellular Ca^{2+} channels. Cocaine may also activate phospholipase C, which hydrolyzes phosphoinositol biphosphate (PIP_2) yielding inositol trisphosphate (IP_3) and diacylglycerol (DAG), which in turn promotes translocation of PKC to the membrane, enhancing its activation. The IP_3 can release Ca^{2+} from internal storage sites, and thus increase intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$). The high levels of Ca^{2+} may also stimulate Ca^{2+} -dependent proteases, degrade membranes and inhibit translation and transcription. Some or all of the above proposed mechanisms may be implicated in cocaine-induced apoptosis, observed at higher doses of cocaine. Although the mechanisms by which cocaine increases Ca^{2+} levels remains to be established, it is attractive to speculate that just like ethanol, cocaine may also increase the number and function of Ca^{2+} channels in the neural PC 12 cell

line.³⁸ Of course, cocaine may also be acting by mechanisms independent of the voltage-dependent Ca^{2+} influx.

In summary, this part of the study showed that cocaine differentially altered the expression of PKC isoforms accompanied by increased levels of Ca^{2+} and total PKC enzyme activity. It is suggested that the differential expression of PKC isozymes may demonstrate distinct roles of PKC isoforms in the actions of cocaine. Thus the PC 12 cell model may be exploited to further understand the neurobiology of cocaine's action in neural systems. Future studies will look at the effect of ibogaine in its own right on the expression and activity of PKC isoforms, then determine if it will reverse cocaine's effects on the activity and expression of PKC isoforms.

CONCLUSION

It is now recognized that addiction is a chronic relapsing brain disease,⁴⁸ for which behavioral and effective safe treatment is urgently needed. Unfortunately, effective drug-abuse treatment continues to be elusive, and efficacies of new treatments for drug and alcohol abuse have been largely disappointing. The discovery that ibogaine, an emerging controversial potential treatment for alcohol and drug addiction along with the recognition that the mesocorticolimbic dopamine may not, after all, underlie the reward pathway as previously hypothesized may facilitate and aid rapid progress in substance abuse research beyond the nucleus accumbens and dopamine hypothesis of reward. As reported recently, since ibogaine's excitatory effect on ventral tegmental area (VTA) neurons was not long-lasting, nor does it persistently alter cocaine- or morphine-induced changes in dopamine neuron impulse,⁴⁹ we concluded that other mechanisms must be explored to account for the proposed antiaddictive properties of ibogaine. For the *in vitro* studies we report that cocaine disrupts signal transduction in PC 12 cells by altering the expression and activity of PKC isoforms and Ca^{2+} levels. Since cocaine differentially altered the expression of PKC isoforms accompanied by increased levels of Ca^{2+} and total PKC activity, it remains to be determined if ibogaine will block the effects of cocaine on the expression of PKC isozymes and activity. For the *in vivo* studies it was demonstrated that acutely, ibogaine induced an aversive behavior in the ICR mice in the plus-maze. Subacutely ibogaine induced variable responses and fluctuations in the plus-maze test. Ibogaine did not by itself precipitate withdrawal anxiogenesis in the mouse model, but reversed the withdrawal aversions caused by cessation from cocaine administration. Therefore, it was concluded that if anxiety is a factor in drug dependency, then the antiaddictive property of ibogaine *in vivo* may be associated with modifying the CNS neurotransmission that may be involved in anxiety.

The ability of ibogaine to alter drug-seeking behavior may thus be due to combined actions of the parent drug and metabolite at key pharmacological targets that modulate the activity of drug reward circuits. Thus further studies are required to establish the efficacy of ibogaine and the design of ibogaine-like compounds for substance abuse treatment that lack the toxicity and hallucinogenic profile of Ibogaine.

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